

**REMARKS/ARGUMENTS**

Claims 1-30 and 32 are pending, claims 25-30 have been withdrawn. Claims 1-24 and 32 stand rejected for reasons of record. Applicants request reconsideration. Applicants respond to the office action using the paragraph numbering of the office action.

5. Restriction requirement

The Examiner maintains the restriction requirement on the basis that the Choo et al. reference is relevant to the claimed invention. This presents essentially the same issues as the rejections under 35 USC 102 and 103. If applicants prevail on these issues, then it follows that Choo et al. is not detrimental to unity of invention and the restriction requirement should be withdrawn.

6-7. The office action repeats the previous rejections under 35 USC 102 and 35 USC 103. Applicants maintain traverse for reasons of record and below.

8. The office action replies to the responses of March 11, 2003 and April 10, 2003, in which applicants provide an example showing that applying the presently claimed quadruplet rules and the triplet rules of Choo et al. can lead to different results. The office action says that the triplet code of Choo et al. corresponds to the first three bases of the present quadruplet code, and the fourth base of the quadruplet code corresponds to the first base in the next series of codes. The office action alleges that if base 4 of the quadruplet code is redefined as base 1 of the next triplet then the results are the same. Thus, in applicants' example in which a T occurs in position 4 and a G in position 1, the office action alleges that if base 4 were renumbered to base 1 and thus represented by a T, the results are the same. Applicants disagree with this analysis.

In essence, the office action appears to be reverting to the position that any difference between the quadruplet and triplet codes is merely an illusion which disappears on some renumbering of the bases. This is not the case. In fact, the triplet code does not correspond

to the first three bases of the present quadruplet code. Rather, as shown in the Table previously provided to the Examiner (another copy of which is attached), the triplet code corresponds to bases 2, 3 and 4 of the quadruplet code. Further, overlap exists only between adjacent quadruplets, not between adjacent triplets. Such is illustrated by the figure attached to the response of March 5, 2003. Thus, base 4 of the quadruplet bound by zinc finger 1 is also base 1 of the immediately adjacent quadruplet bound by zinc finger #2. However, in the triplet code base 4 (in the parlance of the quadruplet code) is the 3' base of the triplet bound by zinc finger #1 and does not occur in the triplet encoding zinc finger #2 at all. Thus, there is no reason to number the base 4 "T" as base 1 in the triplet code. Also, there is no base 1 in the triplet code or rule to apply to it. Thus, the allegation of the office action that a renumbering of bases when applying triplet rules results in the same design as following the claimed quadruplet design rules is wrong.

That the triplet and quadruplet rules can lead to different results is perhaps most simply appreciated by comparing the length of the respective target sequences, and the number of nucleotides taken into account in the design. In the triplet code, each zinc finger binds a triplet subsite, so the target sequence has  $3n$  nucleotides, where  $n$  is the number of zinc fingers in the zinc finger protein. In a quadruplet code, a single zinc finger binds to 4 nucleotides of the target sequence. Two zinc fingers with overlapping quadruplet subsites bind a combined target sequence of seven nucleotides. Three zinc fingers with overlapping quadruplet subsites binds to 10 nucleotides, and  $n$  zinc fingers with overlapping quadruplet subsites bind to a target sequence with  $3n+1$  nucleotides. Thus, in each instance, an extra target nucleotide is used in the design of a zinc finger protein by the quadruplet code, compared to the triplet code. In general, the additional information from the extra nucleotide in the target sequence leads to improved designs of zinc finger proteins.

In previous responses, applicants have referred to design of a single zinc finger to illustrate the difference between triplet and quadruplet codes. The comparison is perhaps easier to appreciate when considering the design of multi-finger zinc finger proteins. Applicants attach two figures to this response (Exhibits A and B) showing the design of two three-finger zinc

finger proteins applying both the triplet rules of Choo et al. and the presently claimed quadruplet rules. In Exhibit A, the design obtained using the triplet code is shown at the top and the design obtained using the claimed quadruplet code at the bottom of the figure. The target sequence used in the quadruplet design is the sequence AGCGGGTGCG with the ten bases numbered in that order. The target sequence used in the triplet design is the same except that the first A is not included. As discussed above, it is inevitable that the target sequence used for triplet design has at least one fewer nucleotide than the target sequence used for quadruplet design. The triplet design is obtained by applying the design rules of Choo et al. to the three triplets GCG, GGT and GCG. The quadruplet design is obtained by applying the present quadruplet rules to the quadruplets AGCG, GGGT and TGCG. The +2 position in each finger is specified by a different nucleotide in the target sequence depending whether the triplet or the claimed quadruplet design rules is followed. Consequently, the +2 position in each finger is occupied by a different amino acid depending on whether triplet or the claimed quadruplet design rules are applied.

Exhibit B compares triplet and quadruplet designs for a different target sequence. Again, the design obtained using the triplet code is shown at the top and the design obtained using the quadruplet code at the bottom of the figure. The target sequence used in the quadruplet design is the sequence TCCATCTGCC with the ten bases numbered in that order. The target sequence used in the triplet design is the same except that the first T is not included. Again, the target sequence used for triplet design has one fewer nucleotide than the target sequence used for quadruplet design. The triplet design is obtained by applying the design rules of Choo et al. to the three triplets CCA, TCT and GCC. The quadruplet design is obtained by applying the present quadruplet rules to the quadruplets TCCA, ATCT and TGCC. It can be seen that different results occur for the +2 and +6 positions in finger 1, the -1 and +2 positions of finger 2, and the +2 and +6 positions of finger 3 (shaded). In the case of finger 2, the +2 position is specified by different nucleotides in the target sequence. The above figures unequivocally show that that the triplet and quadruplet design schemes can result in different zinc finger protein designs, and that the different designs are not simply an artifact of different numbering systems.

Applicants reiterate previous remarks that the patentability of a design method should be judged from the steps of the method recited in the claims rather than from the nature of the zinc finger protein resulting from the method. An improved method of design is not obvious over a prior method of design simply because the improved method of design might result in some of the same proteins as the prior method. An improved method of design that has different method steps than a prior method may allow design of some of the same proteins as a prior method, but in addition will also allow design of some proteins that would not result from following the prior method. If the steps in the improved methods are not suggested by those of the prior method, then the improved method is patentable notwithstanding that it may design some proteins that are the same as those from the prior method.

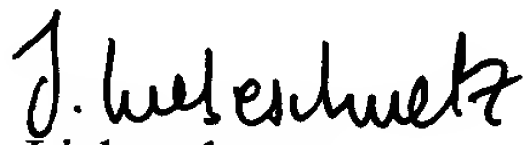
Each of the present claims specifies a process of selecting a quadruplet of nucleotides in a target nucleic acid and then applying design rules to the quadruplet. As has been shown, this procedure can result in different designs than following the triplet design rules discussed in Choo et al. Because Choo et al. does not discuss or suggest selecting a quadruplet of nucleotides or the consequences of doing so, it is submitted that the present claims are neither anticipated nor rendered obvious by Choo et al. either alone or in combination with Krizek.

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Amdt. dated August 13, 2003  
Amendment under 37 CFR 1.116 Expedited Procedure  
Examining Group 1653

PATENT

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 650-326-2400.

Respectfully submitted,

  
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60018127 v1



Exhibit A

N

C

F1				F2				F3			
-1	Arg			-1	Arg			-1	Arg		
+2	Asp			+2	Asp			+2	Asp		
	(specified by G <sup>2</sup> )				(specified by G <sup>5</sup> )				(specified by G <sup>8</sup> )		
+3	Asp, Leu,			+3	His			+3	Asp, Leu,		
	Thr, Val								Thr, Val		
+6	Arg			+6	Ser, Thr			+6	Arg		
	3'	M	5'		3'	M	5'		3'	M	5'
5'-	T	C	G	C	C	C	A	C	G	C	-3'
3'-	A <sup>1</sup>	G <sup>2</sup>	C <sup>3</sup>	G <sup>4</sup>	G <sup>5</sup>	G <sup>6</sup>	T <sup>7</sup>	G <sup>8</sup>	C <sup>9</sup>	G <sup>10</sup>	-5'
	1	2	3	4							
				1	2	3	4				

-1	Arg
+2	Arg, Gln, <b>not</b> Asp (specified by A <sup>1</sup> )
+3	Ser, Glu, Asp, Leu, Thr, Val
+6	Arg, Lys

F1

-1	Arg
+2	Ser, Thr (specified by T <sup>7</sup> )
+3	Ser, Glu, Asp, Leu, Thr, Val
+6	Arg, Lys

F3

-1	Arg
+2	Glu (specified by G <sup>4</sup> )
+3	His
+6	Ser, Thr, Val, Lys

F2

N

C

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Exhibit B

N

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F1		F2		F3	
-1	Asp	-1	Gln	-1	Arg
+2	Not specified	+2	Ser (specified by T <sup>5</sup> )	+2	Asp (specified by T <sup>7</sup> and G <sup>8</sup> )
+3	Asp, Leu, Thr, Val	+3	Asp, Leu, Thr, Val	+3	Asp, Leu, Thr, Val
+6	Not specified	+6	Ser, Thr	+6	Not specified

		3'	M	5'	3'	M	5'	3'	M	5'	
5'-	A	G	G	T	A	G	A	C	G	G	-3'
3'-	T <sup>1</sup>	C <sup>2</sup>	C <sup>3</sup>	A <sup>4</sup>	T <sup>5</sup>	C <sup>6</sup>	T <sup>7</sup>	G <sup>8</sup>	C <sup>9</sup>	C <sup>10</sup>	-5'
	1	2	3	4							
				1	2	3	4				
								1	2	3	4

-1	His, Asp
+2	Ser, Thr (Specified by T <sup>1</sup> )
+3	Ser, Glu, Asp, Leu, Thr, Val
+6	Glu, Asn, Val

F1

-1	Arg
+2	Ser, Thr (specified by T <sup>7</sup> )
+3	Ser, Glu, Asp, Leu, Thr, Val
+6	Ser, Thr, Val, Ala, Glu, Asn

F3

-1	His, Thr
+2	Arg, Gln (specified by A <sup>4</sup> )
+3	Ser, Glu, Asp, Leu, Thr, Val
+6	Val, Lys, Ser, Thr

F2

N

C



		<b>WO 96/06166</b> <b>(Choo et al. cited)</b>	<b>09/424,487</b> <b>(Attorney 006220)</b>
<b>5'</b> <b>(base 4)</b>	<b>G</b>	Arg +6 Ser +6, Asp ++2 Thr +6, Asp ++2	Arg +6 Lys +6
	<b>A</b>		Glu +6 Asn +6 Val +6
	<b>T</b>	Ser +6/Asp ++2 Thr +6/Asp ++2	Ser +6 Thr +6 Val +6 Lys +6
	<b>C</b>		Ser +6 Thr +6 Val +6 Ala +6 Glu +6 Asn +6
<b>mid</b> <b>(base 3)</b>	<b>G</b>	His +3	His +3
	<b>A</b>	Asn +3	Asn +3
	<b>T</b>	Ala +3 Ser +3 Val +3	Ala +3* Ser +3 Val +3
	<b>C</b>	Asp +3 Leu +3 Thr +3 Val +3	Ser +3 Asp +3 Glu +3 Leu +3 Thr +3 Val +3
<b>3'</b> <b>(base 2)</b>	<b>G</b>	Arg -1/Asp +2	Arg -1
	<b>A</b>	Gln -1/Ala +2	Gln -1
	<b>T</b>	Asn -1 Gln -1/Ser +2	His -1 Thr -1
	<b>C</b>	Asp -1	Asp -1 His -1

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3' of 3' (base 1)	G		Glu +2
	A		Arg +2 Gln +2
	T		Ser +2 Thr +2
	C		Asn +2 Gln +2 Arg +2 His +2 Lys +2

\* small residue at -1 or +6

\*\* ++2 is not Asp

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